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Antimicrobial Activity and Metabolite Profiles of *Begonia multangula* Blume from Cibodas Botanical Garden and Gunung Halimun Salak National Park, Indonesia

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ABSTRACT. *The resistance of microorganisms requires innovation in finding for alternative natural substances with antimicrobials properties. One of the plants reported to have antimicrobial potential is the plant Begonia multangula Blume. The purpose of this study was to examine the antimicrobial activity and metabolite profiles of ethanol extracts from the leaf and petiole of B. multangula originating from Cibodas Botanical Garden (KRC) and Gunung Halimun Salak National Park (TNGHS). Ethanol extracts of B. multangula leaf and petiole from KRC and TNGHS at a concentration of 100 mg/ml were tested for their antimicrobial activity against the growth of Staphylococcus aureus ATCC 25923 and Candida albicans using the disk diffusion method. Tetracycline 100 mg/ml and fluconazole 50 mg/ml were used as positive controls, and aquadest as a negative control. The metabolite profile compounds were obtained by GC-MS analysis. The results of the study showed that the highest antimicrobial activity was found in the leaf extract of B. multangula from KRC, with an average inhibition zone of 13,5 ± 1,73 mm against S. aureus and 10 ± 0,08 mm against C. albicans, which categorized as moderate. The most highly detected antimicrobial metabolites in the leaf and petiole extracts of B. multangula from KRC were Hexadecanoic acid ethyl ester. Furthermore, the major compound found in the B. multangula from TNGHS, was Neophytadiene and n-Hexadecanoic acid in leaf and petiole. These findings suggest that B. multangula has the potential to be developed as a natural antimicrobial agent in the future*

Keywords: *antimicrobial, Begonia multangula, bioprospecting, GC-MS*

INTRODUCTION

Microorganisms such as bacteria and fungi are among the leading causes of infectious diseases that continue to evolve over time. Infections caused by microorganisms growing within the body, such as bacteria, viruses, parasites, and fungi, can lead to fatal conditions. Microorganisms such as bacteria and fungi that often cause infections are *Staphylococcus aureus* and *Candida albicans.*

Staphylococcus aureus is a gram-positive bacterium cause for infections found on human skin and the respiratory tract [1]. *Staphylococcus aureus* that has become resistant to methicillin antibiotics is called Methicillin-resistant *Staphylococcus aureus* (MRSA). It has been reported that the resistance to MRSA continues to increase over time. Resistance is the ability of microorganisms to survive despite being exposed to antimicrobial agents that are typically effective in killing or inhibiting their growth.

In 2019, a study was conducted on the prevalence of MRSA at Dr. Soeradji Tirtonegoro General Hospital in Klaten, which found an increase in MRSA prevalence from 7.69% in 2015 to 12.94% in 2018 [2]. Globally, in Indonesia were 34,500 deaths caused by antimicrobial resistance (AMR) and 133,800 deaths associated with AMR in 2019 [3].

In addition to bacteria, infections can also be caused by other microorganisms such as the fungus *Candida albicans*. This fungus can cause a condition known as candidiasis. Candidiasis is a disease affecting the mucous membranes of the mouth, vagina, and gastrointestinal tract [4]. In the United States, it is estimated that there are around 25.000 cases of candidemia, which is a bloodstream infection caused by *Candida* each year [5]. According to data from the Indonesian Ministry of Health, the rate of antibiotic resistance in candidiasis cases in Indonesia reaches 25-50% [6].

In the face of the growing threat of microbial resistance, it is necessary to finding an alternative natural subtance as antimicrobial agents. The use of natural subtance has been increasingly developed in line with advancements in plant bioprospecting research. Natural antimicrobial agents derived from plants include active compounds such as flavonoids, tannins, saponins, and terpenoids. [7].

One such plant reported to contain active compounds such as flavonoids, tannins, saponins, and terpenoids is *Begonia multangula* Blume. *Begonia multangula* is one of Indonesia's endemic plant species from the Begoniaceae family that can not only serve as an ornamental plant, but also has the potential to be developed as a medicinal plant [8]. The habitus of *B. multangula* is an erect herb with hairy petioles that are red or dark green, oval leaves with an asymmetrical heart-shaped base and pointed tips. Its inflorescence type is an umbel-like compound [9] (Figure 1).

Figure 1. (A) Leaves ; (B) petiole and flowers *of Begonia multangula* Blume at Cibodas Botanical Garden

Begonia multangula is commonly found in the moist forests of Java and Sumatra. In West Java, *B. multangula* can be found in the ex situ conservation area, namely the Cibodas Botanical Garden (KRC), located at the foot of Mount Gede and Mount Pangrango. *Begonia multangula* is also found in the in situ conservation areas such as Gunung Halimun Salak National Park (TNGHS). Local communities in the Gunung Halimun Salak National Park area utilize the leaves of *B. multangula* as a fresh vegetable (*lalapan*) and the petioles as a substitute for tamarind or to remove the fishy smell in fish, as well as for medicinal purposes [9].

The abundance of *B. multangula* in both ex situ conservation areas, such as KRC and in situ conservation areas, such as TNGHS, along with the underdeveloped information regarding its metabolite content and potential, has piqued researchers' interest for further study. Sample collection from these two different locations was conducted to compare the metabolite compounds and antimicrobial capabilities of samples growing in ex situ conservation areas (KRC) with those growing in in situ conservation areas (TNGHS). Different growing locations, such as altitude, can result in variations in temperature, humidity, light intensity, rainfall, and nutrient content [10]. Environmental condition differences can affect the bioactive compound content in plants, which may influence their antimicrobial activity [30].

Previous research conducted by Putri, *et. al.* [11] demonstrated the antibacterial activity of *B. multangula* leaf and petiole extracts against *Porphyromonas gingivalis* (gram-negative bacteria), with the highest antibacterial activity found in the ethanol extract of the petiole. Meanwhile, in a similar study, Sari, *et. al.* [12] tested the ethanol extract of *B. multangula* petiole and found that the extract exhibited very strong antibacterial activity against *Aggregatibacter actinomycetemcomitans* (gram-negative bacteria),

Figure 2. Research Locations: (a) Map of West Java Province, (b) Gunung Halimun Salak National Park, (c) Cibodas Botanical Garden

forming the largest inhibition zone at a concentration of 50%, which was 20,33 mm.

Based on these findings, researchers felt the need for further investigation to test the potential of *B. multangula* leaf and petiole extracts for antimicrobial activity against different bacteria such as *S. aureus* (gram-positive bacteria) and the fungus *C. albicans*. Additionally, Gas Chromatography-Mass Spectrometry (GCMS) analysis was performed to identify the metabolite profile compounds in *B. multangula* that have the potential to act as antimicrobial agents.

RESEARCH METHODS

Sample Preparation

The sample preparation steps involve collecting the leaf and petiole of *B. multangula* from the plant conservation areas of Cibodas Botanical Garden and Gunung Halimun Salak National Park (Figure 2). Environmental parameters such as air temperature, humidity, light intensity, soil pH, and soil relative humidity were measured during sample collection. Subsequently, 200 grams each of leaf and petiole were cleaned and air-dried on newspaper. This was followed by oven drying at a temperature of 40°C. Once dried and reaching a constant weight, the samples were ground into powder using a blender.

Extraction Process

The extraction was carried out using the maceration method. The samples were macerated with 99% P.A ethanol solvent at a sample-to-solvent ratio of 1:10. The maceration process was performed by homogenizing the extract on a shaker rotator for at a temperature of 20^{o}C for 24 hours. Subsequently, the filtrate and residue were filtered using Whatman No.1 filter paper to obtain a crude extract. The crude extract was then placed into 1,5 ml tubes and stored at a temperature of 4°C for further analysis.

Qualitative Phytochemicals Assay

A number of ethanol extracts of *B. multangula* leaf and petiole were placed into test tubes for phenol, flavonoid, alkaloid, tannin, and saponin tests.The qualitative phytochemical test used the methods with modifications.

Tannin Test : 3 drops of extract were added with 5 drops of hot water and 5 drops of 1% $FeCl_3$. The presence of tannins was indicated by a color change to blackish green [13].

Phenol Test : 5 drops of extract were added with 10 drops of 1% $FeCl_3$. A color change to blue or bluish-green indicated the presence of phenolic compounds[14].

Flavonoid Test : The test tube containing 4 drops of extract was heated for 5 minutes. Then, 3 drops of ethanol were added, followed by 1 spatula of magnesium powder and 5 drops of HCl. A positive flavonoid result was indicated by a color change to yellow, red, or orange [13].

Saponin Test : 3 drops of extract were added with 7 drops of hot water and then cooled. The mixture was shaken vigorously for 10 minutes. After 10-15 minutes, stable foam indicated the presence of saponins. The presence of saponins was confirmed if the foam remained after adding 1N HCl [14].

Alkaloid Test : 3 drops of extract were added with 3 drops of 1N HCl and 7 drops of water, then heated in a water bath for 2 minutes. Subsequently, 2 drops each of Bouchardat's, Mayer's, and Dragendorff's reagents were added. A positive result was indicated by the presence of a white precipitate when Mayer's reagent was added, an orange-brown color with Dragendorff's reagent, and a brownish-black precipitate with Bouchardat's reagent.

Antimicrobial Activity Test of *Begonia multangula* Blume. Extract

The antimicrobial test was conducted using an agar disk diffusion method or Kirby-Bauer. The extracts were diluted with ethanol to concentration of 100 mg/mL. Aquadest was used as a negative control. 100 mg/mL Tetracycline served as a positive control for antibacterial drug, and 50 mg/mL Fluconazole as a positive control for antifungal drug.

a. Antibacterial Activity Test

A total of 100 µl of *Staphylococcus aureus* ATCC 25923 at a density of 4,5 x 10^6 was inoculated into 10 ml of physiological saline solution (0,9% NaCl). Subsequently, transfer 100 µl of the bacterial isolate onto the surface of the nutrient agar (NA) medium using a micropipette. Then, using the spread plate method, spread the suspension with a sterilized L-glass rod. Each leaf extract (KRC and TNGHS) and petiole extract (KRC and TNGHS) with a concentration of 100 mg/mL, each with a volume of 100 µl, was added to a blank disk (6 mm diameter) and inoculated on the media. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones was observed.

b. Antifungal Activity Test

A total of 100 µl of *Candida albicans* at a density of 2,7 x 10^6 was inoculated into 10 ml of physiological saline solution (0,9% NaCl). Subsequently, transfer 100 µl of the fungal isolate

onto the surface of the potato dextrose agar (PDA) medium using a micropipette. Then, using the spread plate method, spread the suspension with a sterilized L-glass rod. Each leaf extract (KRC and TNGHS) and petiole extract (KRC and TNGHS) with a concentration of 100 mg/mL, each with a volume of 100 µl, was added to a blank disk (6 mm diameter) and inoculated on the media. The plates were incubated at 37°C for 24 hours, and the diameter of the inhibition zones was observed.

GC-MS Analysis

The metabolite profile of ethanolic extracts of leaf and petiole of *B. multangula* from KRC and TNGHS were observed by GC-MS. Metabolite compound analysis was conducted using Agilent 7890B gas chromatography (GC) equipped with a 5977A mass spectrometer (MS). The column used was Agilent 19091S-433: 93.92873 DB-5MS UI (5% Phenyl Methyl Silox.) ((325 °C): 30 m x 250 µm x 0,25 µm). Helium was used as the carrier gas. The injector temperature was set at 40°C, and the detector temperature at 300°C. The initial temperature was maintained for 1 minute. A 1 µL sample was injected for analysis. The resulting chromatogram was identified by comparing the mass spectrum with library data (NIST 11 Library and Wiley Library) and the Pubchem website for GC retention times against known standards [62].

Data Analysis

Antimicrobial activity data were analyzed by measuring the clear zones around the paper disks and calculating the standard deviation of the mean inhibition zone diameters across four replicates. The average inhibition zone diameters were then interpreted based on the classification by Jarriyawattanachaikul *et. al.* [15] (Table 1) to determine the inhibition potency category of the test solution. Subsequently, the inhibition zone diameter data were statistically analyzed using the ANOVA (Analysis of Variance) method in SPSS software and post hoc analysis with the DMRT (Duncan's Multiple Range Test) at a significance level of 0,05 to determine significant differences among the data. Meanwhile, metabolite profile compound analysis was conducted using GC-MS and recorded in Microsoft Excel, with comparisons made to the Pubchem and NIST websites.

Table 1. Inhibition Potency Classification.

Inhibition Zone Diameter (mm)	Inhibition Response	Symbol
1- 8 mm	Weak	+
9 – 14 mm	Moderate	++
15 – 19 mm	Strong	+++
≥ 19 mm	Very Strong	++++

RESULTS AND DISCUSSIONS

Environmental Parameters for Sample Collection and Sample Preparation

Samples of *B. multangula* were taken from the KRC (*ex situ*) in West Java. Sample collection was carried out during the day in clear weather, with environmental air temperature measurements at 23,3°C ± 1,07, air humidity at 70% ± 0,57, and light intensity at 1860 lux. Topographically the KRC area is a highland with an elevation of 1211 – 1437 meters above sea level, characterized by low temperatures and a wet climate [16].

The second sampling location was in its natural habitat (*in situ*), namely Gunung Halimun Salak National Park (TNGHS) in West Java. TNGHS is a tropical mountain rainforest area with three types of ecosystems: lowland rain forest at elevations of 500 - 1000 meters above sea level, sub-montane forest at elevations of 1000 - 1500 meters above sea level, and montane forest at elevations of 1500 - 1929 meters above sea level [17]. Sampling in TNGHS was carried out at an elevation of 972 ± 8,62 meters above sea level (lowland rain forest), with a location temperature of 21,8°C ± 1,37, air humidity of 73% ± 7, and light intensity of 7298 lux ± 8397.

Samples of *B. multangula's* leaf and petiole, which were cut and cleaned (Figure 3), dried and ground into powder. The ground samples cause the cells to rupture, facilitating the binding of metabolites by the solvent [18]. The samples were extracted with 99% P.A ethanol with maceration technique for 24 hours. The extract yield percentage shows a higher extraction yield for leaves from KRC, which is 5,096%, compared to leaves from TNGHS, which is 4,38%. Additionally, the extraction yield percentage for petioles from KRC is also higher, at 5,25%, compared to petioles from TNGHS, which is 4,203%.

Figure 3. Sample preparation of *B. multangula* leaf and petiole.

The extraction yield percentage is related to the concentration of bioactive compounds in the plant material. The higher the concentration of

bioactive compounds in a sample, the higher the extraction yield [19]. One factor causing the difference in extraction yield percentages is the growing environment, which affects the content of bioactive compounds in a plant [20].

The soil in KRC is well-maintained and irrigated, providing consistent moisture levels to support the growth of bioactive compounds in *B. multangula*. In contrast, the soil moisture in TNGHS is more variable due to natural forest conditions, with fluctuations in water and nutrient availability affecting the synthesis of the plant's bioactive compounds. Overall, the soil pH at both sampling locations ranges from 5,9 – 6,2, categorizing it as moderately acidic soil. This indicates an optimal soil pH for plant growth at both sampling locations [21].

Qualitative Phytochemical

Phytochemical screening was conducted to determine the content of bioactive compounds in each extract. Qualitative phytochemical testing is a preliminary test to identify the general presence of metabolite profile compounds in the sample by observing color changes and precipitate formation. The results of the phytochemical tests on the ethanol extract of *B. multangula* leaf (Table 2) from KRC showed positive results for phenol, flavonoid, alkaloid, tannin, and saponin. These results differed from the phytochemical tests on the ethanol extract of *B. multangula* leaf from TNGHS, which only showed positive results for phenol, tannin, and saponin.

Meanwhile, the results of the phytochemical tests on the ethanol petiole extract of *B. multangula* from KRC (Table 2) showed positive results for containing flavonoid, alkaloid, tannin, and saponin. On the other hand, phytochemical screening results for the ethanol petiole extract of *B. multangula* from TNGHS showed positive results for containing alkaloid and saponin. The phytochemical screening results of *B. multangula* petiole from KRC are consistent with Sari, *et. al.* [12], where the ethanol extract of *B. multangula* petiole showed positive results for containing flavonoid, tannin, alkaloid, and saponin.

Both phytochemical screening results indicate that samples collected from the ex situ conservation area of Cibodas Botanical Garden (KRC) contain a greater variety of metabolite compounds compared to samples taken from the in situ conservation area (Gunung Halimun Salak National Park). This difference can be attributed to various environmental factors such as temperature, altitude, and soil nutrients between samples collected from different locations [22]. At KRC, the environmental conditions are more controlled and well-maintained, including consistent irrigation. This supports optimal growth and can enhance the production of bioactive compounds in plants.

Phenolic compounds play a role as antibacterial agents through a mechanism of absorption with bacterial cells involving hydrogen bond interactions [23]. Flavonoids are a class of phenolic compounds that can be found in vegetables, fruits, bark, stems, roots, and flowers. Flavonoids have roles as antioxidants, anti-inflammatory, antimutagenic, and antimicrobial agents [24]. Alkaloid compounds can be found in leaves, bark, twigs, and seeds. Alkaloids have roles as antimicrobial compounds and pain relievers [25]. Tannins are a group of polyphenols that act as antibacterial, antioxidant, and pain-relieving agents due to inflammation [26]. Saponins are glycosides divided into steroidal and triterpenoid saponins. The roles of saponin compounds include antiviral, anticancer, hypocholesterolemic, and antimicrobial agents [27].

Table 2. Qualitative Phytochemical Test Results of Ethanol Extracts of *B. multangula* Leaf and Petiole from KRC and TNGHS

Compound Classes	*B. Multangula* Extract			
	Leaf KRC	Leaf TNGHS	Petiole KRC	Petiole TNGHS
Phenol	++	++	-	-
Flavonoid	+	-	++	-
Alkaloid Mayer	+	-	+++	++
Alkaloid Bouchardat	+	-	+	+
Alkaloid Dragendorf	+	-	+	+
Tannin	++	+	+	-
Saponin	++	+	++	+

Note: (-) = negative, (+) = weakly positive, (++) = strongly positive, (+++) = very strongly positive.

Antimicrobial Activity of B. multangula Extract

The results of the antimicrobial activity test against *S. aureus* and *C. albicans* are shown in Table 3. The antimicrobial activity assay was divided into 6 groups, including K+, K-, K1, K2, K3, and K4 (notes are below Table 3). The concentration of extract used was 100 mg/ml. From the test results against *S. aureus*, the group with the highest inhibitory power was the K+ (tetracycline 100 mg/mL) with an average inhibition zone of 50 ± 0,08 mm. Then among the B. multangula extract samples, the leaf extract from KRC (K1) showed the highest inhibitory activity against the test microorganisms *S. aureus*

and C. albicans compared to other extracts (Table 3).

According to the inhibition response classification table by Jarriyawattanachaikul *et. al.* [15], the positive control (tetracycline) can be categorized as very strong in inhibiting the growth of *S. aureus.* Tetracycline has a broad spectrum that can inhibit the growth of gram-positive bacteria by inhibiting the binding of aminoacyl-tRNA to the ribosomal acceptor, thereby hindering the elongation of the amino acid chain (bacterial protein) [28]. The ethanol leaf extract from KRC (13,5 ± 1,79 mm) is considered moderate, the ethanol petiole extract from KRC (10 ± 0,08 mm) is considered moderate, the ethanol petiole extract from TNGHS (8 ± 0,43 mm) is considered weak, and the ethanol leaf extract from TNGHS and the negative control (aquadest) do not have activity that inhibits the growth of *S. aureus*.

Table 3. Inhibition Zone Diameter of *B. multangula* KRC and TNGHS

Test Microbes	Extract Groups	Mean Inhibition Zone Diameter (mm)
S. aureus	K +	$50 \pm 0{,}08^{a\,(++++)}$
	K -	$0 \pm 0^{e\,(-)}$
	K 1	$13{,}5 \pm 1{,}79^{b\,(++)}$
	K 2	$10 \pm 0{,}08^{c\,(++)}$
	K 3	$0 \pm 0^{e\,(-)}$
	K 4	$8 \pm 0{,}43^{d\,(+)}$
C. albicans	K +	$9 \pm 0{,}14^{b\,(++)}$
	K -	$0 \pm 0^{d\,(-)}$
	K 1	$10 \pm 0{,}08^{a\,(++)}$
	K 2	$4 \pm 0{,}16^{c\,(+)}$
	K 3	$0 \pm 0^{d\,(-)}$
	K 4	$0 \pm 0^{d\,(-)}$

Note : (abcde) Different superscript letters within the same column indicate significant differences ($p < 0{,}05$). (-) no inhibitory activity, (+) weak inhibitory activity, (++) moderate inhibitory activity, (++++) very strong inhibitory activity.

K+ = Positive Control (Tetracycline 100 mg/ml and Fluconazole 50 mg/ml)
K - = Negative Control Group (Aquadest)
K1 = *B. multangula* Leaf Ethanol Extract from KRC
K2 = *B. multangula* Petiole Ethanol Extract from KRC
K3 = *B. multangula* Leaf Ethanol Extract from TNGHS
K4 = *B. multangula* Petiole Ethanol Extract from TNGHS

The results of this study are in line with Putri, *et. al.* [11], who also used samples of *B. multangula* leaves and petioles from the KRC collection, showing the ability to inhibit gram-negative bacteria (*Porphyromonas Gingivalis*) with an average inhibition zone range of 6,07 – 10,17 mm. Additionally, the study by Sari, *et. al.* [12] showed that the petiole extract of *B. multangula* has inhibitory activity against gram-negative bacteria (*Aggregatibacter actinomycetemcomitans*) with an average inhibition zone of 13,09 mm at all concentrations. This indicates that *B. multangula* from KRC grows well and contains complex bioactive compounds to inhibit the growth of both gram-positive and gram-negative bacteria.

The largest antifungal inhibition against *C. albicans* was observed in the ethanol leaf extract from KRC (10 ± 0,08 mm) with a moderate category, followed by the positive control (Fluconazole 50 mg/ml) (9 ± 0,14 mm) with a moderate category, and the ethanol petiole extract from KRC (4 ± 0,16 mm) with a weak category. Meanwhile, the negative control (aquadest), ethanol leaf extract from TNGHS and ethanol petiole extract from TNGHS did not produce any inhibition or antifungal activity against *C. albicans.* This may be due to the bioactive compounds in the extracts from TNGHS being less complex compared to the samples from KRC.

The results of the data analysis on the diameter of the inhibition zones against *S. aureus* bacteria and *C. albicans* fungi, conducted using the One-Way ANOVA test, showed a significance value of 0,00 , which means the p-value is less than 0,05. A p-value less than 0,05 indicates a significant difference among the various extract groups in this study. This demonstrates that the positive control and the extract groups from KRC leaf extract, KRC petiole extract, TNGHS leaf extract and TNGHS petiole extract exhibited activities that inhibited the growth of *S. aureus* bacteria and *C. albicans* fungi. The Post Hoc (Duncan) test results also showed significant differences in each tested extract group (Table 3).

The Duncan test results showed that the diameter of the inhibition zones for *S. aureus* bacterial growth in the positive control group significantly differed from the KRC leaf extract group, the KRC petiole extract group, the TNGHS leaf extract group, and the TNGHS petiole extract group. The KRC leaf extract group demonstrated significant differences in all trials, as did the KRC petiole extract group and the TNGHS petiole extract group. In addition to showing significant differences among all extract groups, the KRC leaf extract also exhibited the highest activity. However, the TNGHS leaf extract and negative control groups did not show significant differences because these extract groups did not produce inhibition zones as a form of antibacterial activity.

As for the diameter of the inhibition zone for the growth of the fungi *C. albicans*, the positive control group significantly differed from the

variations of the KRC leaf extract group, KRC petiole extract group, TNGHS leaf extract group, and TNGHS petiole extract group. The KRC leaf extract group showed significant differences compared to all extract variations, as did the KRC petiole extract group. However, the TNGHS leaf and petiole groups, as well as the negative control, did not show significant differences because these extracts did not form an inhibition zone, indicating no antifungal activity in these extract groups. The KRC leaf extract group showed the highest antifungal activity against *C. albicans.*

The larger the inhibition zone formed, the stronger the antimicrobial activity of the compound [15]. Differences in antimicrobial activity are influenced by the active compound content in the sample. Active compounds such as saponins, tannins, flavonoids, and phenols can damage cell membranes, deactivate enzymes, and denature proteins, causing the cell walls of microorganisms to be damaged and leading to cell death [29]. This is consistent with the research results that show differences in antimicrobial activity between *B. multangula* samples from KRC and TNGHS. The results of the phytochemical tests also indicate that samples from KRC have a more varied metabolite content compared to samples from TNGHS (Table 3).

Overall, in this study, the repetition in various *B. multangula* extract variations showed antimicrobial activity (antibacterial and antifungal) with the formation of inhibition zones. This proves the hypothesis in this study that there is a difference in the inhibitory ability between the two samples grown in KRC and TNGHS against the growth of *S. aureus* and *C. albicans.* This significant difference can be attributed to the higher phytochemical content such as phenols, flavonoids, alkaloids, tannins, and saponins that act as antimicrobials in samples from KRC compared to samples from TNGHS. The higher phytochemical content in samples from KRC is due to more optimal environmental and growth conditions that support the production of secondary metabolites. One factor influencing the production of secondary metabolites is temperature stress [30]. In this case, the KRC region has a higher temperature compared to TNGHS. Other factors affecting secondary metabolites include temperature, humidity, light, water availability, and nitrogen availability in the soil. These secondary metabolites are formed as an adaptive response of plants to unfavorable environmental conditions [31].

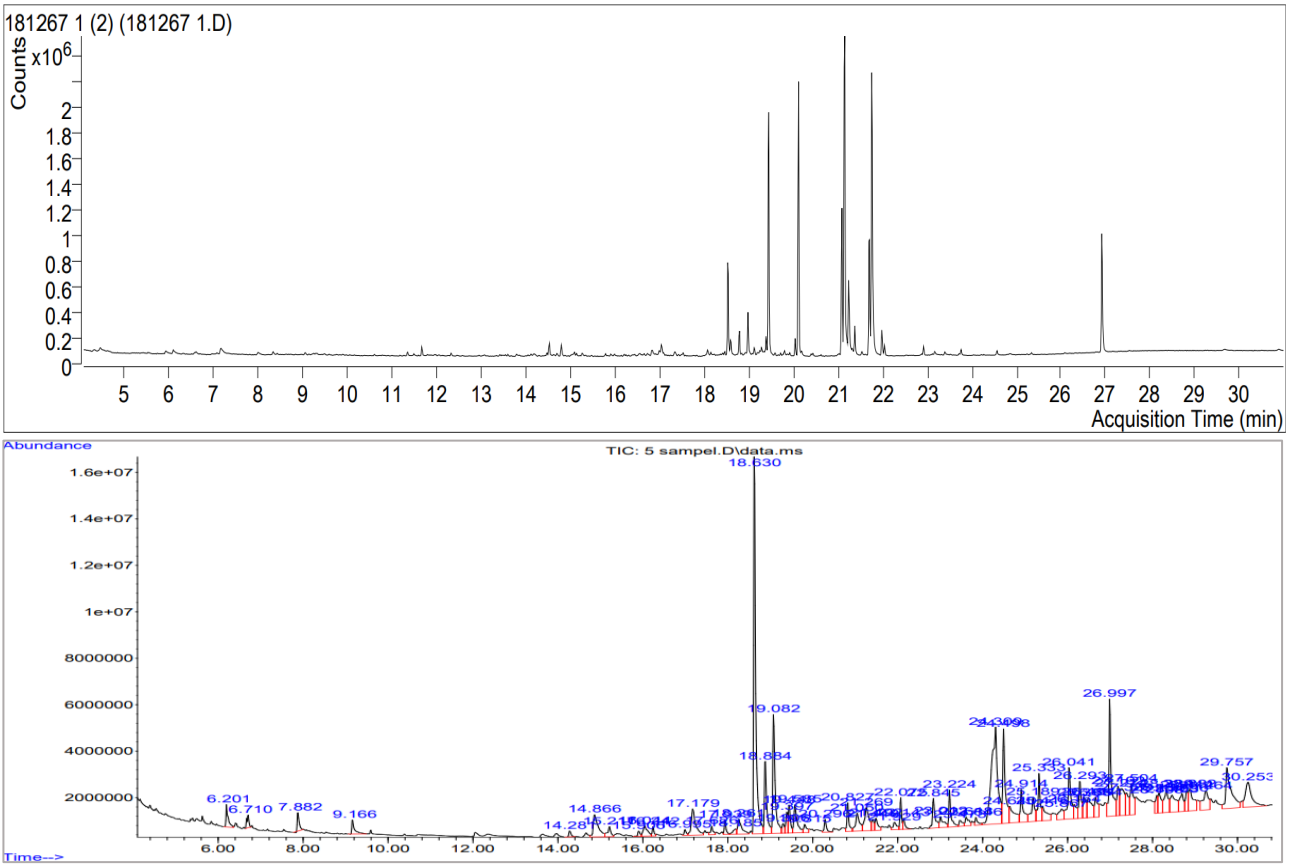

Figure 4. GC-MS Chromatogram of *B. multangula* Ethanol Extract: (A) Leaf from KRC; (B) Leaf from TNGHS

Metabolite Profiles of B. multangula Leaf and Petiole Extract from KRC and TNGHS

Compounds such as phenol, flavonoid, alkaloid, tannin, and saponin detected in *B. multangula* (Table 3) might indicate potential for antimicrobial activity. Compounds like phenol, flavonoid, alkaloid, tannin, and saponin can disrupt microbial cell membrane stability, leading to cell damage [32]. Based on this potential, metabolite content was subsequently checked using GC-MS (Gas Chromatography and Mass Spectrometry) analysis on the leaf and petiole extracts of *B. multangula* to identify compounds contributing to their antimicrobial activity. Using gas chromatography coupled with mass spectrometry, volatile components, hydrocarbons, alcohols, acids, and esters can be accurately

Table 4. Peak Compounds Detected from GC-MS of *B. multangula* Leaf Ethanol Extract from KRC

RT	Area	Match Score	Compound Name	Group	Biological Potential
18.534.6	1055593	86.9	*1-Octadecyne*	Hydrocarbon compounds	Antimicrobial [40]
18.533.7	1181462	95.9	*Neophytadiene*	Terpenoid (Diterpenoid)	Antimicrobial [36]
19.445.7	2682309	84.7	*Decanoic acid, methyl ester*	Fatty acid	Antimicrobial [41]
19.446.3	3174801	96.8	*Hexadecanoic acid, methyl ester*	Fatty acid	Antimicrobial [35]
20.116.8	3185358	96.3	*Hexadecanoic acid, ethyl ester*	Fatty acid	Anti- inflammatory, Antimicrobial [42]
21.086.7	1402192	87.9	*11,14-Eicosadienoic acid, methyl ester*	Fatty acid	Antibacterial [43]
21.087.4	1476191	88	*(Z,Z)-12,15-Octadecadienoic acid methyl ester*	Fatty acid	Antioxidant, Anti- inflammatory [34]
21.147.1	4732726	94.4	*9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-*	Fatty acid	Antioxidant, Anti- inflammatory [34]
21.241.4	1006708	92.4	*Phytol*	Terpenoid (Diterpenoid)	Antioxidant, Antimicrobial, Anti- inflammatory, Antinociceptive [44]
21.700.5	1597876	92.9	*Linoleic acid ethyl ester*	Fatty acid	Anti- inflammatory, Antioxidant [45]
26.913.9	1626386	94.8	*Squalene*	Terpenoid (Triterpenoid)	Antimicrobial, Antioxidant, Antidiuretic [38]

Table 5. Peak Compounds Detected from GC-MS of *B. multangula* Leaf Ethanol Extract from TNGHS

RT	Area Percentage	Match Score	Compound Name	Group	Biological Potential
14.866	1.78	98	*Pentadecane*	Hydrocarbon compounds	Anti- inflammatory, Analgesic, Antipyretic [46]
17.179	1.87	97	*Heptadecane*	Hydrocarbon compounds	Antimicrobial, Antioxidant [47]
18.261	0.98	98	*Octadecane*	Hydrocarbon compounds	Antimicrobial [48]
18.630	12.24	97	*Neophytadiene*	Terpenoid (Diterpenoid)	Antimicrobial [36]
19.585	1.46	98	*Hexadecanoic acid, methyl ester*	Fatty acid	Antimicrobial [35]
21.269	1.61	99	*11-Octadecenoic acid, methyl ester*	Fatty acid	Antioxidant, Antimicrobial [49]
24.640	1.50	93	*Tetrapentacontane, 1,54-dibromo-*	Hydrocarbon compounds	Antimicrobial, Antioxidant, Anticancer, Antidiabetic [50]
26.997	4.35	99	*Squalene*	Terpenoid (Triterpenoid)	Antimicrobial, Antioxidant, Antidiuretic [38]
27.274	2.00	87	*2-Dodecen-1-yl(-)succinic anhydrid*	Fatty acid	Antibacterial [51]
29.757	3.50	97	*6-Methoxy-2,7,8-trimethyl-2-(4,8,1 2-trimethyltridecyl)chroman*	Tokoferol (Vitamin E)	Antioxidant [39]

identified [33]. The chromatogram and GC-MS analysis results of the ethanol leaf extract *B. multangula* collected from KRC are shown in Figure 4 (A) and Table 4. The chromatogram and GC-MS analysis results of the ethanol leaf extract of *B. multangula* from TNGHS are shown in Figure 4 (B) and Table 5.

The GC-MS analysis of ethanol leaf extract of *B. multangula* from KRC revealed 11 compounds with the largest peak areas (Table 4). These compounds include fatty acids, hydrocarbons, and terpenoids. According to the analysis in Figure 4 (A), the three most dominant peak compounds were identified with area values of 4.732.726; 3.185.358; and 3.174.801. The compounds are *9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)-* with a retention time of 21,14 minutes, *Hexadecanoic acid, ethyl ester* with a retention time of 20,11 minutes, and *Hexadecanoic acid, methyl ester* with a retention time of 19,44 minutes. *9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z)-* with the molecular formula $C_{19}H_{32}O_2$ is a fatty acid compound with potential antioxidant and anti-inflammatory properties [34]. *Hexadecanoic acid, ethyl ester* ($C_{18}H_{36}O_2$) and *Hexadecanoic acid, methyl ester* ($C_{17}H_{34}O_2$) are also fatty acid derivatives with potential antimicrobial properties [35].

Meanwhile, the GC-MS results for the ethanol leaf extract of *B. multangula* from TNGHS showed 10 compounds with the largest peak areas (Table 5). These compounds include fatty acids, hydrocarbons, terpenoids, and vitamin E. Based on Figure 4 (B), the three most dominant peak compounds are: *Neophytadiene* with an area percentage of 12,24% and a retention time of 18,63 minutes; *Squalene* with an area percentage of 4,35% and a retention time of 26,99 minutes; and *6-Methoxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl)* chroman with an area percentage of 3,50% and a retention time of 29,75 minutes.

Neophytadiene (C_2OH_{38}) is a diterpenoid compound that is a derivative of terpenoids [36]. Most terpenoid derivatives act as antimicrobials, antibiotics, toxins, and growth regulators [37]. *Squalene* ($C_{30}H_{50}$) is a triterpenoid compound with potential antibacterial, antioxidant, and diuretic properties [38]. *6-Methoxy-2,7,8-trimethyl-2-(4,8,12-trimethyltridecyl) chroman* ($C_{29}H_{50}O_2$) is a derivative of tocopherol (vitamin E). Tocopherol or vitamin E compounds play a significant role as antioxidant [39].

The GC-MS analysis of the ethanol petiole extract of *B. multangula* collected from KRC showed 10 compounds with the largest peak areas

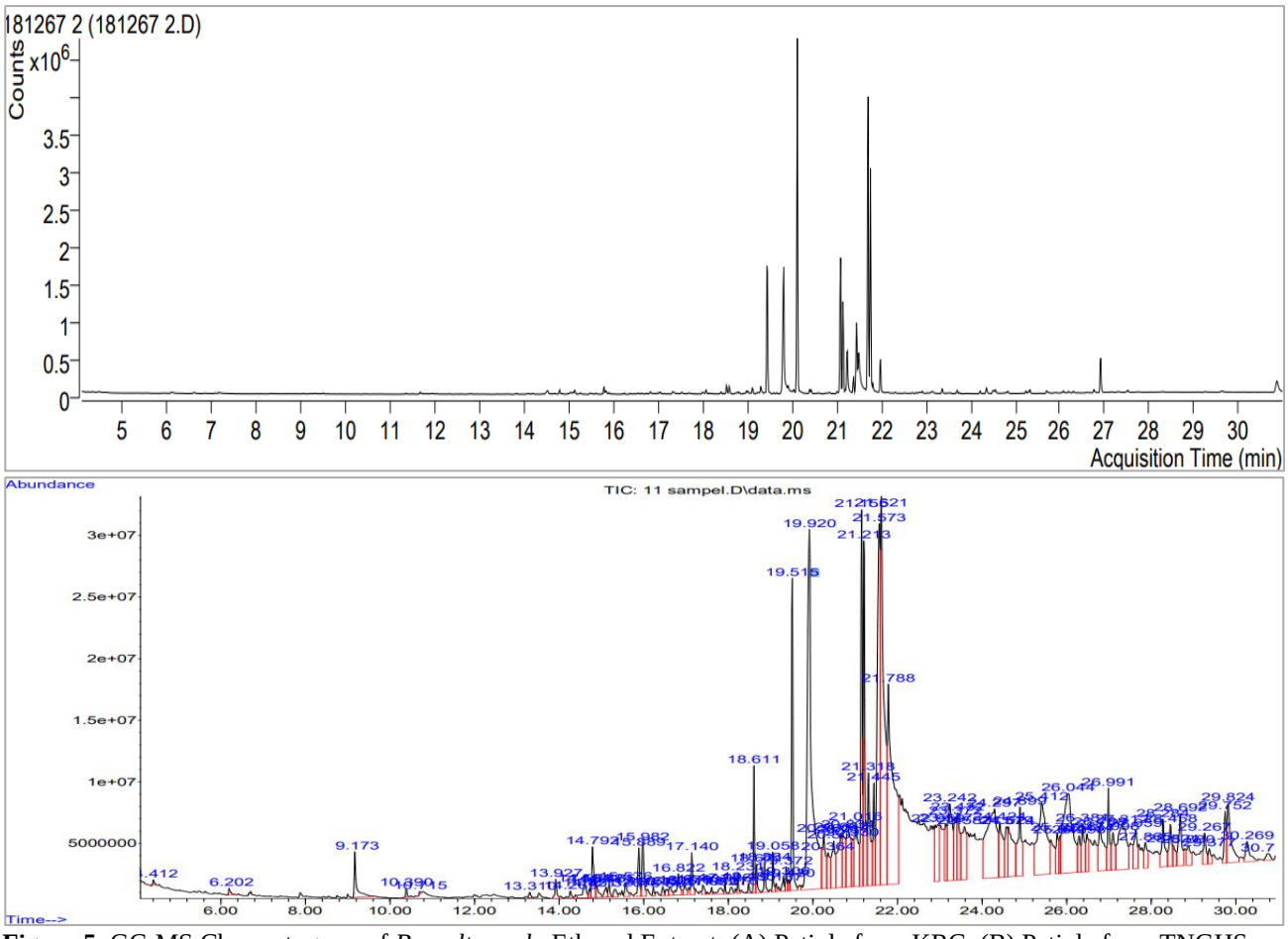

Figure 5. GC-MS Chromatogram of *B. multangula* Ethanol Extract: (A) Petiole from KRC; (B) Petiole from TNGHS

(Table 6). These compounds consist of fatty acids and terpenoids. Based on the analysis in Figure 5 (A), the three most dominant peak compounds are *Hexadecanoic acid, ethyl ester* with an area of 6.695.061 at a retention time of 20,11 minutes; *Linoleic acid ethyl ester* with an area of 5.822.128 at a retention time of 21,7 minutes; and *n-Hexadecanoic acid* with an area of 3.522.291 at a retention time of 19,8 minutes. *Hexadecanoic acid, ethyl ester* ($C_{18}H_{36}O_2$) is a fatty acid compound also found in the GC-MS analysis of *B. multangula* leaf extract from KRC. *Linoleic acid ethyl ester* ($C_{20}H_{36}O_2$) is a fatty acid compound. *n-Hexadecanoic acid* ($C_{16}H_{32}O_2$) is a fatty acid compound with potential antibacterial properties. This is demonstrated in the research by Ganesan, T. *et. al.* [52], showing that *n-Hexadecanoic acid* exhibits antibacterial activity against *S. aureus, B. subtilis, E. coli,* and *K. pneumonia,* indicating its potential as an antioxidant and antibacterial agent.

Meanwhile, the GC-MS analysis of the ethanol petiole extract of *B. multangula* from TNGHS revealed 11 compounds with the largest

Table 6. Peak Compounds Detected from GC-MS of *B. multangula* Petiole Ethanol Extract from KRC

RT	Area	Match Score	Compound Name	Group	Biological Potential
19.442.1	2311268	86.3	*Decanoic acid, methyl ester*	Fatty acid	Antimicrobial [41]
19.442.5	3008258	95.9	*Hexadecanoic acid, methyl ester*	Fatty acid	Antimicrobial [35]
19.811.7	3522291	95.4	*n-Hexadecanoic acid*	Fatty acid	Antibacterial, Antioxidant [52]
20.116.8	6695061	96.6	*Hexadecanoic acid, ethyl ester*	Fatty acid	Anti- inflammatory, Antimicrobial [42]
21.088.4	2616521	94.2	*9,11-Octadecadienoic acid, methyl ester, (E,E)-*	Fatty acid	Antimicrobial, Antioxidant, Anticancer [53]
21.1406	1556235	92.4	*11,14,17-Eicosatrienoic acid, methyl ester*	Fatty acid	Antioxidant, Antimicrobial [54]
21.233.8	1072438	91.7	*Phytol*	Terpenoid (Diterpenoid)	Antioxidant, Antimicrobial, Anti-inflammatory, Antinociceptive [44]
21.701.4	1072438	91.7	*9,12-Octadecadienoic acid (Z,Z)-*	Fatty acid	Antibacterial [55]
21.705.6	5822128	96.6	*Linoleic acid ethyl ester*	Fatty acid	Anti-inflammatory, Antioxidant [45]
26.906.5	706102	87.4	*Squalene*	Terpenoid (Triterpenoid)	Antimicrobial, Antioxidant, Antidiuretic [38]

Table 7. Peak Compounds Detected from GC-MS of *B. multangula* Petiole Ethanol Extract from TNGHS

RT	Area Percentage	Match Score	Compound Name	Group	Biological Potential
19.920	9.32	99	*n-Hexadecanoic acid*	Fatty acid	Antibacterial, Antioxidant [52]
20.717	0.96	91	*Stigmasterol*	Steroid (Fitosterol)	Antimicrobial [56]
21.155	3.35	99	*9,12-Octadecadienoic acid (Z,Z)-, methyl ester*	Fatty acid	Anti-inflammatory, Anticancer [57]
21.318	1.68	98	*Phytol*	Terpenoid (Diterpenoid)	Antioxidant, Antimicrobial, Anti-inflammatory, Antinociceptive[44]
21.445	1.00	99	*Heptadecanoic acid, 14-methyl-, methyl ester*	Fatty acid	Antioxidant, Antimicrobial [54]
21.573	5.68	99	*10E,12Z-Octadecadienoic acid*	Fatty acid	Antibacterial [58]
21.788	7.09	92	*Linoelaidic acid*	Fatty acid	Anticancer, Anti-inflammatory [59]
24.899	1.44	94	*Bis(2-ethylhexyl) phthalate*	Ester asam flatat	Antibacterial [60]
26.044	4.40	99	*4,22-Stigmastadiene-3-one*	Steroid	Antimicrobial [61]
26.991	0.88	99	*Squalene*	Terpenoid (Triterpenoid)	Antimicrobial, Antioxidant, Antidiuretic [38]
29.824	1.37	93	*dl-.alpha.-Tocopherol*	Vitamin E	Antioxidant [39]

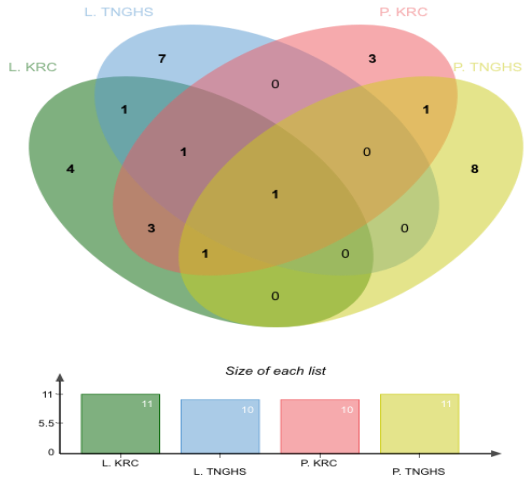

Figure 6. Metabolite Profile Compounds of Leaf and Petiole Extracts of *B. multangula* from KRC and TNGHS. **Note : L. KRC** (Leaf *B. multangula* from KRC); **L. TNGHS** (Leaf *B. multangula* from TNGHS); **P.KRC** (Petiole *B. multangula* from KRC); **P.TNGHS** (Petiole *B. multangula* from TNGHS).

peak areas (Table 7). These compounds consist of fatty acids, terpenoids, steroids, phthalic acid esters, and vitamin E. Based on the chromatogram analysis (Figure 5B), the three most dominant peak compounds are*: n-Hexadecanoic acid* with an area percentage of 9,32% at a retention time of 20,11 minutes; *Linoelaidic acid* with an area percentage of 7,09% at a retention time of 21,7 minutes; and *10E, 12Z- Octadecadienoic acid* with an area percentage of 5,68% at a retention time of 19,8 minutes. *Linoelaidic acid* is a fatty acid compound that acts as an anti-inflammatory and anticancer agent [59]. The compound *10E, 12Z- Octadecadienoic acid* belongs to the group of conjugated linoleic acids (CLA) with potential antibacterial properties [58]. *n-Hexadecanoic acid* is a fatty acid with potential antimicrobial properties [52].

There are three metabolite compounds found in both the leaf extract from KRC and the leaf extract from TNGHS, namely *Neophytadiene ; Hexadecanoic acid, methyl ester* and *Squalene* (Figure 6). In the petiole extract from KRC and TNGHS, three metabolite compounds were also found, namely *n-Hexadecanoic acid* ; *Phytol* and *Squalene*. Samples collected from KRC (leaf and petiole) contain 6 common compounds, namely *Phytol ; Hexadecanoic acid, methyl ester ; Decanoic acid, methyl ester; Hexadecanoic acid, ethyl ester ; Linoleic acid, ethyl ester* and *Squalene*. Meanwhile, leaf and petiole from TNGHS were found to contain one common compound, namely *Squalene.* The metabolite compound detected in all extracts (leaf and petiole from both KRC and TNGHS) is *Squalene*, which belongs to the terpenoid group.

CONCLUSION

Based on this research, the extracts of *B. multangula* from the Cibodas Botanical Garden contain a higher variety of bioactive compounds such as phenols, flavonoids, alkaloids, tannins, and saponins. These compounds exhibit significant antimicrobial activity against *S. aureus and C. albicans*, as evidenced by the formation of inhibition zones. Phytochemical screening and GC-MS analysis identified key bioactive compounds with antimicrobial potential, including *9,12,15-Octadecatrienoic acid, methyl ester*, *Hexadecanoic acid, ethyl ester*, *Hexadecanoic acid, methyl ester*, *Neophytadiene,* and *Squalene*. Environmental factors and growth conditions significantly influence the metabolite composition and yield of bioactive compounds in *B. multangula*. This study supports the hypothesis that ethanol extracts of *B. multangula* from both conservation areas possess antimicrobial properties, with samples grown in ex situ (KRC) environments showing a broader spectrum of bioactive compounds and higher inhibitory activities. These findings underscore the potential of *B. multangula* as a source of natural antimicrobial agents and highlight the influence of habitat on the plant's metabolite profile. However, this study has limitations not using concentration variations for each extract sample tested. Future research can focus on conducting further biological activity tests on bioactive compounds as antimicrobial agents and developing them into pharmaceutical products.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest related to this research.

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